FORMULATION, DEVELOPMENT AND EVALUATION OF RESVERATROL LOADED MICROCARRIER SYSTEM FOR THE MANAGEMENT OF ULCERATIVE COLITIS

SYNOPSIS OF THE THESIS

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DECLARATION

I hereby declare that the dissertation synopsis entitled "**Formulation, Development and Evaluation of Resveratrol Loaded Microcarrier System for the Management of Ulcerative Colitis**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associateship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

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1 Introduction: Ulcerative Colitis

Inflammatory bowel diseases (IBD) is chronic idiopathic relapsing inflammatory disease of the gastrointestinal tract (GIT) tract, Generally IBD is divided into two categories, Ulcerative colitis (UC) and crohn's disease (1). In case of crohn's disease inflammation can occur in any part of digestive system (Mouth to anus), but in case of UC inflammation occurs only in ascending or descending parts of colon. UC is chronic and relapsing disease of the colon, characterized by inflammation with ulcer formation in the lining of colon. A basic symptom of ulcerative colitis includes abdominal pain, blood in stool, weight loss, fatigue and severe pain in the rectum (2). Severe form of ulcerative colitis leads to colon cancer. Chronic inflammation generated by the production of reactive oxygen species leads to generate dysplasia, which further turns into colitis associated colorectal cancer (3). Therefore, there is the high risk of colon cancer in patients with ulcerative colitis (4).

2 Morbidity and mortality of ulcerative colitis

The incidence of UC from Punjab was investigated by cluster sampling method. A population of 51,910 people was screen, among which two thirds lived in rural parts of Punjab and the rest in urban parts. Overall, 23 patients were diagnosed with UC leading to a prevalence rate of 44.3/100,000. These data again indicate that UC is not rare in India (5). However, the crude overall incidence of IBD, UC, and CD from Asia was 1.37, 0.76, and 0.54 per 100,000, respectively, whereas in Australia it was 23.67, 7.33, and 14.00, respectively. Among the Asian countries, it was highest in mainland China (Guangzhou: 3.4/ 100,000) followed by Hong Kong (3.06/100,000), and Macau (2.2/100,000). Data from Western Asia including studies from Kuwait, Turkey and Israel. Revealed an incidence rate of 2.8/100,000 and 5.04/100,000 in Kuwait and Israel, respectively, and a prevalence of 4.9/100,000 and 167/100,000 in Turkey and Israel, respectively (6, 7). All around the globe, UC is turning out to be a global disease, because of increasing incidences and prevalence's of it with respect to time.

2.1 Conventional treatment strategies for ulcerative colitis

The treatment of Ulcerative Colitis depends upon the severity of disease. The most commonly used drugs for its treatment are anti-inflammatory agents which mainly include 5-amino salicylates like mesalazine, olsalazine and balsalazide, which are used for the cure of mild to

moderate attacks and to maintain remission in UC. Some immunosuppressive agents such as azathioprine, 6-mercaptopurine, methothexate, cyclosporine, and tacrolimus are routinely prescribed by doctors. Other classes of drugs which can also be used are corticosteroids, like prednisolone and anti-TNF-α-antibodies for moderate to severe conditions of Ulcerative Colitis (8).

2.2 Selection of drug: Glucocorticoids like Beclomethasone are also used for the treatment of UC but cause weight gain and also affecting the immunity system. The previous data suggested that Resveratrol is good choice of drug for the management of ulcerative colitis (9). Resveratrol is a naturally occurring polyphenol that exhibits pleiotropic health beneficial effects including anti-inflammatory, cardio- and cancer-protective activities. It is recognized as one of the more promising natural molecules in the prevention and treatment of chronic inflammatory and autoimmune disorders (9-12). Resveratrol mixed in food ameliorates DSS induced colitis in mice in a dose-dependent manner. Resveratrol significantly improves inflammation score, down regulates the percentage of neutrophils in the mesenteric lymph nodes and lamina propiria, and modulates $CD3^+$ T cells that express tumor necrosis factor-alpha and interferon gamma. Markers of inflammation and inflammatory stress are also down regulated by resveratrol (13).

3 Polymer selections:

Proposed polymers:

3.1 Coated polymer:

3.1.1 Cellulose acetate phthalate (CAP): CAP is a commonly used polymer phthalate in the formulation of pharmaceuticals, such as coating of tablets or capsules and for controlled release formulations. It prevents the release of drug from acidic environment (14).

3.2 Enzyme based polymer:

3.2.1 Pectin:

Pectin, a natural heteropolysaccharide, is characterized with 1,4 linked a-D-galactosyluronic acid residues and neutral sugars such as rhamnose, galactose and arabinose. It is popularly used as colon- specific drug delivery vehicle for colon disease. It has positive effect on ulcerative colitis disease state. Pectin can be selectively digested by colonic microflora to induce site-specific drug release, and has a strong tendency to interrupt the colon tumor growth biochemically. The pectin is an aqueous soluble polymer and it undergoes partial degradation at gastric pH 2–4 via side

chain hydrolysis and at small intestinal pH 5–6 via b-elimination of main chain or de-esterification (15), (16).. The matrix of pectin tends to swell, erode and has premature drug release at upper gastrointestinal tract. The colon- specific oral dosage forms are designed chiefly through coating of drug matrix with Cellulose acetate phthalate.

3.3 Proposed strategies:

3.3.1 Coated and enzyme based strategies

We will prepare pectin based formulation (micro particles and pellets) followed by coating with CAP. The Coated CAP will prevent the drug release in stomach. Furtherly, at pH in the range of 6.0 to 7.4 CAP layer will dissolve and come in contact with colon's specific enzymes (Bacteroids Bifidobacterium, Eubacterium) and start releasing the at inflammed colon site. The above said strategy has chosen to avoid burst drug and probiotic release in the stomach. In case of Ulcerative colitis, these is a slight variation of colonic pH , but due to specific enzyme based approach slight variation in pH does not affect the release rate of drug and probiotic from pectin based formulation.

3.4 Proposed carriers for colon drug delivery:

3.4.1. Microparticle as carrier:

The microparticle is polymer matrix with an active moiety dispersed inside free flowing spherical particles. The active ingredients in the matrix is usually dissolved or melted in polymer before processing. It provides constant and prolonged therapeutic effect. Microparticle carrier system has the ability to provide specific uptake in certain areas, such as absorption in the colon, in order to target inflammation sites in UC. The particle size of microparticle is ranging from 1-1000 μm. It is necessary to develop a colon-specific delivery system for resveratrol so that drug can be targeted to inflammation site of colon. Microparticles could be best approach for colon targeting. The particle size 5–15 μm of enzyme based microparticle provides localization of drug, ultimately increases the residence time of drug in the colonic region. Particle size of this range is able to adhere at the site of inflammation, finally provide maximum release of drug at colonic pH with maximum pharmacological action (17).

3.4.2 Pellets as carrier:

Pellets are solid, small free flowing spherical units, prepared by agglomeration of fine powder called pellets. Pellets range in size typically, between 0.5 – 1.5 mm, though other sizes could be prepared. Bulking agents and binders are required for preparation of pellets by extrusion and spheronization method. We are using HPMC as a binder, MCC as a bulking agent and CAP as coating polymer for colon targeted drug delivery. CAP will prevent the drug release from gastric acidic medium. Polysaccharides polymers such as guar-gum, pectin are used in colon targeted drug delivery systems due to its drug release retarding property and susceptibility to microbial degradation in large intestine. The drug and polysaccharide polymer with necessary diluent will be allowed to form pellets by extrusion and spheronization method (18).

3.4.3 Reason for preparing both microparticle and pellets as carrier: The composition of microparticle and pellets are different. Their *in-vivo* effect with respect influence of drug release and *in-vivo* drug targeting might be different which may further influence therapeutic efficacy.

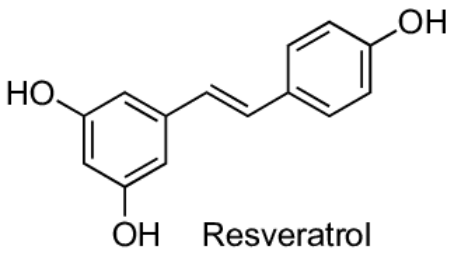
4. Literature review:

- Elizaberth Managalia *et al*. (19) Suggest that Mesalamine, 5-aminosalicylic acid (5-ASA), is a potent antioxidant and is known to enhance peroxisome proliferator–activated receptor activity in the intestine. Reactive oxygen species in colonic progenitor cells and enhances PTEN (Phosphatase and tensin homolog) activity, thus attenuating PI3K/Akt signaling. These data suggest that the antioxidant properties of 5-ASA may be the predominant mechanism for 5-ASA chemoprevention.
- Nidhi *et al.* (20) has developed embelin loaded guar gum microparticles through emulsification technique. The in vitro release of the optimized formulation was found to be 88.5±3.8% in 24 hr, have exhibit remarkably sustain and delayed the release of embelin at a specific site. Embelin loaded microparticles showed average particle size of 12.9± 0.75 µm. This approach produced comparatively less side-effect to another conventional dosage form. The drug release was site-specific and sustained. This approach produced less side-effect as compare to another conventional dosage form. The study examined the application of the microparticulate system in enhancing stability and controlled release.
- Seema Badhana *et al*.,(21) prepared microspheres by the ionic-gelation emulsification method using tripolyphosphate (TPP) as cross linking agent. The microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach. It was observed that Eudragit S-100 coated chitosan microspheres gave maximum release of drug in the colonic environment. Eudragit-coated chitosan microspheres were promising carriers for colon-targeted delivery of Mesalamine.
- Ankita Sharma *et al.* (22) has developed embelin loaded guar gum microparticles through emulsification technique. The in vitro release of the optimized formulation was found to be 88.5±3.8% in 24 hr, have exhibit remarkably sustain and delayed the release of embelin at a specific site. Embelin loaded microparticles showed average particle size of 12.9± 0.75 µm.This approach produces comparatively less side-effect to another conventional dosage form. The drug release is site-specific and sustained. This approach produces less side-effect as compare to another conventional dosage form. The study

examined the application of the microparticulate system in enhancing stability and controlled release.

- Varshosaz J *et al.*(23) has prepared colon-specific pellets of budesonide, using pectin as film coating. Pellet cores of budesonide were prepared by extrusion / spheronization technique. Pectin, in different ratios was combined with Eudragit RS30D, Eudragit NE30D or Surelease to produce film coating. The dissolution profiles of pectin coated pellets were investigated in pH of 1.2 (2 h), pH of 7.4. (4 h) and pH of 6.8 in the absence as well as presence of rat cecal contents (18 h). This confirmed the ability of the optimized formulation for targeted drug delivery of budesonide to colon.
- Sureshkumar R *et al.* (24) High molecular weight hydroxypropyl methylcellulose (HPMC) and biodegradable pectin were used for coating the pellets containing curcumin, to be released in the colon. The prepared pellets were free flowing. *In-vitro* release of curcumin remained intact up to pH 3.0, disintegrated at pH 7.2, and released up to 12 hours. The ideal batch (1:3) showed minimum release at pH 1.2 and maximum release at pH 6.8, and an increased amount of curcumin in the blood stream(1.287 µg/ml) was achieved when compared with pure curcumin (0.5 µg/ml)
- Fan L *et al.* (25) investigated the physiochemical characteristics and preventive effects of purified pectin (H121, L13 and L102) with different esterification degrees on dextran sulfate sodium (DSS)-induced colitis in mice. Three doses of each type of pectin were administered to C57BL/6J mice for 7 days before the DSS treatment, with dextran and Mesalazine as positive controls. The results suggested that pectin could attenuate DSS-induced intestinal epithelial injury, inflammation and oxidative stress.

5.Drug Profile:



Resveratrol

Molecular Formula: $C_{14}H_{12}O_3$

Molecular Weight: 228.24

Chemical Name: 3,5,4'-trihydroxy-trans-stibene

Solubility: 50g/l(ethanol)

BCS Classification : Class2(low solubility and high permeability)

6 Aim: The main aim of the current study is to design, develop and evaluate resveratrol containing delivery systems to deliver the API at colon site to ensure optimum anti-inflammatory activity and achieve increased therapeutic effectiveness.

Research objective: In order to achieve the aim of this research, the following objectives are proposed:

1. To develop and optimize micro carrier system/ formulations of resveratrol.
2. To optimize critical variables (materials) for development of microcarriers formulations.
3. To characterize the developed carrier systems (in vitro).
4. To carry out accelerated stability studies of the optimized formulations.
5. To evaluate *in vivo* efficacy of developed formulations.
6. To study kinetic model.

7. Plan of work

1. Literature Review
2. Preformulation Studies of Resveratrol
   - Physical appearance
   - Purity & identification
     - UV spectroscopy
     - FTIR spectroscopy
     - Melting Point
     - Solubility Analysis
     - Partition Coefficient
   - Development of Analytical method by UV spectroscopy.
3. Preparation and optimization of microparticles by DOE & RSM
4. *In-vitro* characterization of optimized formulation of microparticles
   a) Loading efficiency
   b) Drug entrapment efficiency
   c) Particle morphology (SEM)
   d) Particle size analysis, polydispersity index
   e) Zeta potential
   f) *In-vitro* drug release
   g) Storage stability studies
   h) Kinetic Model

5. *In-vitro* characterization of optimized formulation of pellet

   a) Loading efficiency
   b) Drug entrapment efficiency
   c) Particle morphology (SEM)
   d) Sphericity studies(optical microscopic)
   e) Micrometric properties (Angle of repose, Bulk density, Tapped density, Hausner's ratio, Carr's index and friability.
   f) *In-vitro* drug release
   g) Storage stability studies

h) Kinetic Model

6. *In-vivo* studies using TNBS model induced ulcerative colitis model in Wistar rat

a) Morphological analysis :study various parameter like colon injury ,inflammation, gross appearance of colon, severity of intestinal injury

b) **Histopathological studies**: In histopathological studies the diagnosis and study of diseases of tissue and involves examining tissue/cell under a microscope.

c) Oxidative stress studies: To study the effect of drug in wistar rat

7. Statistical analysis

8. Compilation of thesis, publication of research outcomes and submission of thesis

References:

1. Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. The Journal of clinical investigation. 2007 Mar 1;117(3):514-21.
2. Rashid M, Kaur V, Hallan SS, Sharma S, Mishra N. Microparticles as controlled drug delivery carrier for the treatment of ulcerative colitis: A brief review. Saudi Pharmaceutical Journal. 2016 Jul 1;24(4):458-72
3. Rogler G. Chronic ulcerative colitis and colorectal cancer. Cancer letters. 2014 Apr 10;345(2):235-41.
4. Navaneethan U, Jegadeesan R, Gutierrez NG, Venkatesh PG, Hammel JP, Shen B, Kiran RP. Progression of low-grade dysplasia to advanced neoplasia based on the location and morphology of dysplasia in ulcerative colitis patients with extensive colitis under colonoscopic surveillance. Journal of Crohn's and Colitis. 2013 Dec 15;7(12):e684-91.
5. Kedia S, Ahuja V. Epidemiology of inflammatory bowel disease in India: the Great Shift East. Inflammatory intestinal diseases. 2017;2(2):102-15.
6. Ray G. Long-term maintenance treatment of ulcerative colitis in eastern India: a 15-year follow-up. Tropical doctor. 2018 Jul;48(3):207-13.
7. Ng SC, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, Wong TC, Leung VK, Tsang SW, Yu HH, Li MF. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. Gastroenterology. 2013 Jul 1;145(1):158-65.
8. Kucharzik T, Asthana AK. Medical Management 2: Conventional. InInflammatory Bowel Disease Nursing Manual 2019 (pp. 81-90). Springer, Cham.
9. Nunes S, Danesi F, Del Rio D, Silva P. Resveratrol and inflammatory bowel disease: the evidence so far. Nutr Res Rev. 2018 Jun;31(1):85-97.
10. Pujara N, Wong KY, Qu Z, Wang R, Moniruzzaman M, Rewatkar P, Kumeria T, Ross BP, McGuckin M, Popat A. Oral Delivery of β-Lactoglobulin-Nanosphere-Encapsulated Resveratrol Alleviates Inflammation in Winnie Mice with Spontaneous Ulcerative Colitis. Mol Pharm. 2021 Feb 1;18(2):627-640.

11. Gandhi H, Rathore C, Dua K, Vihal S, Tambuwala MM, Negi P. Efficacy of resveratrol encapsulated microsponges delivered by pectin based matrix tablets in rats with acetic acid-induced ulcerative colitis. Drug Dev Ind Pharm. 2020 Mar;46(3):365-375.
12. Abdallah DM, Ismael NR. Resveratrol abrogates adhesion molecules and protects against TNBS-induced ulcerative colitis in rats. Can J Physiol Pharmacol. 2011 Nov;89(11):811-8.
13. Iglesias N, Galbis E, Díaz-Blanco MJ, Lucas R, Benito E, de-Paz MV. Nanostructured Chitosan-Based Biomaterials for Sustained and Colon-Specific Resveratrol Release. Int J Mol Sci. 2019 Jan 18;20(2):398.
14. Kapoor D, Maheshwari R, Verma K, Sharma S, Ghode P, Tekade RK. Coating technologies in pharmaceutical product development. In Drug Delivery Systems 2020 Jan 1 (pp. 665-719). Academic Press.
15. Noreen A, Akram J, Rasul I, Mansha A, Yaqoob N, Iqbal R, Tabasum S, Zuber M, Zia KM. Pectins functionalized biomaterials; a new viable approach for biomedical applications: A review. International journal of biological macromolecules. 2017 Aug 1;101:254-72.
16. Kang RK, Mishr N, Rai VK. Guar Gum Micro-particles for Targeted Co-delivery of Doxorubicin and Metformin HCL for Improved Specificity and Efficacy Against Colon Cancer: In Vitro and In Vivo Studies. AAPS PharmSciTech. 2020 Feb;21(2):1-1.
17. Rashid M, Kaur V, Hallan SS, Sharma S, Mishra N. Microparticles as controlled drug delivery carrier for the treatment of ulcerative colitis: A brief review. Saudi Pharmaceutical Journal. 2016 Jul 1;24(4):458-72.
18. Singh MN, Hemant KS, Ram M, Shivakumar HG. Microencapsulation: A promising technique for controlled drug delivery. Research in pharmaceutical sciences. 2010 Jul;5(2):65.
19. Managlia E, Katzman RB, Brown JB, Barrett TA. Antioxidant properties of mesalamine in colitis inhibit phosphoinositide 3-kinase signaling in progenitor cells. Inflammatory bowel diseases. 2013 Jul 17;19(10):2051-60.
20. Nidhi, Dadwal A, Hallan SS, Sharma S, Mishra N. Development of enteric-coated microspheres of embelin for their beneficial pharmacological potential in ulcerative colitis. Artificial cells, nanomedicine, and biotechnology. 2017 Aug 18;45(6):1092-100

21. Badhana S, Garud N, Garud A. Colon specific drug delivery of mesalamine using eudragit S100-coated chitosan microspheres for the treatment of ulcerative colitis. International Current Pharmaceutical Journal. 2013 Feb 1;2(3):42-8.
22. Sharma A, Kaur N, Sharma S, Sharma A, Rathore MS, Ajay K, Mishra N. Embelin-loaded guar gum microparticles for the management of ulcerative colitis. Journal of microencapsulation. 2018 Feb 17;35(2):181-91.
23. Varshosaz J, Emami J, Tavakoli N, Minaiyan M, Rahmani N, Dorkoosh F, Mahzouni P. Pectin film coated pellets for colon-targeted delivery of budesonide: In-vitro/in-vivo evaluation in induced ulcerative colitis in rat. Iranian journal of pharmaceutical research: IJPR. 2012;11(3):733.
24. Sureshkumar R, Munikumar M, Ganesh GN, Jawahar N, Nagasamyvenkatesh D, Senthil V, Raju L, Samantha MK. Formulation and evaluation of pectin-hydroxypropyl methylcellulose coated curcumin pellets for colon delivery. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm. 2014 Sep 24;3(2).
25. Fan L, Zuo S, Tan H, Hu J, Cheng J, Wu Q, Nie SP. Preventive effects of pectin with various degrees of esterification on ulcerative colitis in mice. Food & Function. 2020.